COVANCE.
THE DEVELOPMENT SERVICES COMPANY

Sponsor:

The Procter & Gamble Company
Cincinnati, Ohio

PROTOCOL

Study Title:

21-Day Dermal Toxicity Study with SS0853.01 in Rats

Date:

20 November 2000

Testing Facility:

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704-2595

Laboratory Study Identification:

Proposal 30760A

Covance 6114-398

Sponsor Study Identification:

Procter & Gamble Study No.: DRD: SSBTS00.040-52068

Study
21-Day Dermal Toxicity Study with SS0853.01 in Rats

Purpose
To evaluate the toxicity of the test material when administered daily by topical application to the dorsal skin of rats for at least 21 days

Sponsor
The Procter & Gamble Company
Sharon Woods Technical Center, C1N39H
11530 Reed Hartman Highway
Cincinnati, Ohio 45241

Study Monitor
Jennifer L. Counts, PhD
The Procter & Gamble Company
Telephone No.: 513.626.0023
Facsimile No.: 513.626.3522

Alternate Sponsor Contact
John Wisler, PhD, DABT
The Procter & Gamble Company
Miami Valley Laboratories
11810 East Miami River Road
Cincinnati, Ohio 45252
Telephone No.: 513.627.0992
Facsimile No.: 513.627.1167

Study Location
Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704-2595

Study Director
Susan Henwood, MS, DABT
Covance Laboratories Inc.
Telephone No.: 608.241.7221
Facsimile No.: 608.242.2736

Study Toxicologist
Dennis J. Hensen
Covance Laboratories Inc.
Telephone No.: 608.242.2712, Ext. 2507
Facsimile No.: 608.242.2736

Principal Investigator for Ophthalmology
Stephen Bistner, DVM
Diplomate, ACVO
University of Minnesota
Veterinary Teaching Hospital
1352 Boyd Avenue
St. Paul, Minnesota 55108

Proposed Study Timetable
Experimental Start Date (animal receipt): 13 November 2000
Inlife Start Date: 27 November 2000
Inlife Termination Date: 21 December 2000
Audited Draft Report Date: 10 April 2001
Experimental Termination Date: 10 July 2001

Regulatory Compliance
This study will be conducted in compliance with the Environmental Protection Agency Good Laboratory Practice Regulations as set forth in Title 40 of the United States Code of Federal Regulations, Part 160 (Federal Insecticide, Fungicide, and Rodenticide Act), issued August 17, 1989 (effective October 16, 1989); with the Organisation for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice, ENV/MC/CHEM (98)17; and with any applicable amendments.

Regulatory Guidelines
The study design is based on the United States Environmental Protection Agency Office of Prevention, Pesticides, and Toxic Substances (OPPTS), Series 870, Health Effects Testing Guidelines, No. 870.3200, 21/28-Day Dermal Toxicity, August 1998.

Animal Care and Use Statement
All procedures in this protocol are in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office for Laboratory Animal Welfare. In the opinion of the sponsor and study director, the study does not unnecessarily duplicate any previous work.

Major Computer Systems
The major computer systems to be used on this study may include, but not be limited to, the following systems. The Path/Tox System (PTS) application, supplied by Xybion Medical Systems Corporation, will be used for the direct on-line capture of inlife toxicology and anatomical pathology data. The San Diego Instruments PAS Motor Activity System will be used for collection of motor activity data. The Randomization and Data Extension Systems (RADES) and Automatic Form and Label Generation System (AFLGS) applications will be used in conjunction with the PTS system to randomize animals and produce labels and forms, respectively. The Talisman application will be used for the dose preparation information, and the Millennium system will be used for the collection of the dose analysis data. The Clinical application will be used for the collection of clinical pathology data. The Report Generation System application will be used to transfer the information from PTS and the Clinical application into Word or WordPerfect for reporting purposes. All version numbers of the applications are maintained in the log book for the application.

Quality Assurance
The protocol, study conduct, draft report, and final report will be audited by the Covance Quality Assurance Unit. In addition, at a minimum, the following critical phases will be audited: test material receipt, storage, and handling; dose preparation; dose analysis; dose application and removal; animal observations; expanded clinical observations; body weight; sample collection; necropsy; and any other critical phases specified by Covance Standard Operating Procedures (SOPs).

Test Material

Identification/Lot Number
SS0853.01 (.01 is the lot number)

Purity
Responsibility of, and provided by, the sponsor

Stability
Expiration date: September 20, 2001

Storage Conditions
At ambient conditions

Characteristics
Information on synthesis methods, composition, or other characteristics that define the test material is on file with the sponsor.

Safety
The sponsor will provide relevant occupational safety information known about the test material (e.g., Material Safety Data Sheet, safety instructions, test material identity).

Vehicle

Identification
Reverse osmosis (RO) water

Storage Conditions
At ambient conditions

Reserve (Archive) Samples
A reserve sample of each lot of test material (30 mL) will be taken and stored at ambient conditions.

Disposition of Test Material
After authorization from the sponsor, any remaining test material will be returned to:

Dan S. Stevens
The Procter & Gamble Company
11530 Reed Hartman Highway
C1N17, SWTC
Cincinnati, Ohio 45241
Telephone No.: 513.626.4123
Facsimile No.: 513.626.3522

The recipient will be notified before shipment.

Animals

Species
Rat

Strain
Crl:CD®(SD)IGS BR

Source
Charles River Laboratories, Inc., Portage, Michigan

Age at Initiation of Treatment
Approximately 7.5 to 9 weeks

Weight at Initiation of Treatment
Females: 150 to 225 g
Males: 200 to 300 g

Number and Sex
40 males and 40 females (nulliparous and nonpregnant)

Identification
Implantable microchip identification device with unique identification number

Justification
Rats historically have been used in safety evaluation studies and are recommended by appropriate regulatory agencies.

Husbandry

Housing
Animals will be housed individually in suspended, stainless-steel cages (may be group-housed during acclimation).

Diet
Certified rodent diet (#8728C, Harlan Teklad) *ad libitum*, unless otherwise specified. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Results of specified nutrient and contaminant analyses are on file at Covance-Madison.

Water
Ad libitum. Samples of the water are routinely analyzed for specified microorganisms and environmental contaminants. The results are on file at Covance-Madison.

Contaminants
There are no known contaminants in the diet or water at levels that might interfere with this study.

Environment
Environmental controls for the animal room will be set to maintain 19 to 25°C, a relative humidity of 30 to 70%, and a 12-hour light/12-hour dark cycle. The light/dark cycle may be interrupted for study-related activities.

Acclimation
At least 1 week; all animals will be weighed on the day of arrival.

Randomization
Animals may be eliminated from consideration for selection to the study based on data collected during acclimation. Animals will be assigned to treatment groups using a computerized blocking procedure designed to achieve body weight balance with respect to treatment groups. Prior to assignment to groups, the weight variation of the animals of each sex used will not exceed ±2 standard deviations of the mean weight. After assignment to groups, the mean body weight for each group of each sex will not be statistically different at the 5.0% probability level. At randomization, the coefficient of variation of the mean weight for each sex and group will not exceed 10%.

Environmental Enrichment and Dietary Supplements
Will not be provided

Group Designations and Dose Levels

	No. of Animals		Dose Level[a]	Dose Concentration[a]
Group	Males	Females	(mg/kg/day)	(mg/mL)
1 (Control)	10	10	0	0
2 (Low)	10	10	100	100
3 (Mid)	10	10	500	500
4 (High)	10	10	1,000	1,000[b]

a The dose volume will be 1 mL/kg. Group 1 animals will receive the vehicle only.
b The high-dose will receive the test material neat.

Rationale for Dose Level Selection
The goal of dose selection is a gradient of toxic effects. Signs of toxicity are possible at the high-dose level that was selected per OPPTS 870.3200. The low-dose level is anticipated to be a no-effect level. The mid-dose level was selected as an additional dose for the purpose of evaluating any potential toxicologic effects.

Dosing Procedures

Dose Preparation
Approximately weekly. All dose preparations will be mixed according to the mixing procedure, which will be maintained in the study records. Dose concentrations will be based on the test material as supplied. All dose preparations will be stored at room temperature.

Method of Administration
Topical application to the dorsal skin for at least 21 days at approximately the same time each day, except on days when expanded clinical observations are scheduled. Dosing solutions or vehicle will be applied based on the most recently recorded body weight at a dose volume of 1 mL/kg/dose. Treatment will continue through the day before necropsy. Animals will be treated for a minimum of 6 hours per day (maximum exposure of 7 hours per day; dose duration timing based on the last animal/group). For each exposure, the test material preparation or vehicle will be applied using a syringe or pipette (a new disposable pipette tip will be used for each animal) directly to the back of each animal. During exposure, the test material preparation or vehicle will be held in place with a porous gauze dressing (equal to or less than 8-ply) and nonirritating tape. The application site will be covered with an elastic bandage. The animals will be collared with flexible plastic collars during the exposure to prevent access to the application site. The animals will be acclimated to the collars for 3 to 5 days prior to initiation of dosing.

At the end of each exposure period, the elastic bandage, tape, dressing, and collar will be removed and dose sites will be wiped with reverse osmosis water-moistened paper tissues/towels.

Reason for Dosing Route
The potential route of exposure to humans is dermal.

Application Site
The area of exposure will constitute approximately 10% of the total body surface area (approximately 25 cm^2). The fur will be clipped from the dorsal area of the trunk on the day before initiation of treatment (Day –1) and at approximately weekly intervals thereafter or as deemed necessary (clipping will be done after the 6- to 7-hour exposure period and scoring). Care will be taken to avoid abrading the skin. The exposure area will be centered on the dorsal midline of the animal. Dose sites will be marked with indelible ink as needed.

Retention Samples
Will not be taken

Dose Analysis
Analysis of the dose preparations will be conducted by Covance using an analytical method supplied by the sponsor and validated by Covance. Acceptance criteria for results of individual or mean values is ± 10% of the theoretical concentration.

Homogeneity
Dose levels selected for this study are solutions, therefore, homogeneity testing is not required.

Stability
Two sets of samples (approximately 5 mL each) will be taken from the low- and mid-dose levels mixed pretest and analyzed. One set will be analyzed on the day of mixing. One set will be stored at room temperature for at least 10 days, then analyzed.

Routine Analysis
Samples (approximately 5 mL each) from all dose preparations will be collected and analyzed. Stability samples collected from the mid- and high-dose level preparations and analyzed on the day of mixing will be used for Week 1 routine analyses. All samples will be stored at room temperature until analyzed.

Observation of Animals

Clinical Observations
AM/PM Mortality Observations: Twice daily (a.m. and p.m.), each animal will be observed for mortality and moribundity; findings will be recorded as they are observed.

Routine Clinical Observations: At least once prior to treatment, on the day of initiation of treatment, and weekly during treatment, detailed observations will be made for each animal; abnormal findings (ranked/graded, if appropriate) or an indication that the animal appears normal will be recorded. These weekly observations will be made outside the home cage and will include, but not be limited to, changes in skin, fur, eyes, and mucous membranes; occurrences of secretions and excretions; and autonomic activity (e.g., lacrimation, piloerection, pupil size, or unusual respiratory pattern). Changes in posture and reactivity to handling, the presence of clonic or tonic movements, stereotypes (e.g., excessive grooming, circling), or bizarre behavior (e.g., self mutilation, walking backwards) will also be recorded weekly. Changes in gait will be assessed weekly by allowing the animal to walk freely to allow evaluation of gait. Additional findings will be recorded as they are observed.

Expanded Clinical Observations
A detailed description of the measurements included in the expanded clinical observation evaluations and the scoring criteria used for these measurements is presented in Attachment No. 1. For expanded clinical observations, rats will be tested in random order. Weekly expanded clinical observation testing sessions will occur at approximately the same time of day to limit the possible effects of diurnal variation. The observations will be performed prior to dosing on the days conducted. Random order will be determined using a computerized randomization procedure and the procedure will be documented in the study records. When possible, the same randomization will be used throughout the study. The technicians conducting the expanded clinical observations will be unaware of each animal's dose level.

Hand-held and open-field observations:
Hand-held (Attachment No. 1, Items 1 through 11) and open-field observations (Attachment No. 1, Items 12 through 15) will be performed prior to treatment and weekly during the study. These observations will be done on a day other than that scheduled for the routine clinical observations.

Elicited behaviors observations:
Elicited behaviors observations (Attachment No. 1, Items 16 through 22, including forelimb and hindlimb grip strength and nociceptive reflex) will be evaluated once during the last week of the study. Within two weeks prior to the elicited behavior evaluations, the animals to be tested will be acclimated to the grip strength devices. This acclimation may be performed in one day and does not need to be conducted in random order. The acclimation procedure will be documented.

Motor Activity Testing
Motor activity testing will also be done once during Week 4 (Day 23 or 24). The animals will be placed into an automated photocell activity recording device and activity will be recorded for 40 minutes. Activity counts will be recorded at 2-minute intervals. Testing will be done to include an approximately equal distribution of animals/sex/group/device. Motor activity testing will be done either after enhanced clinical observations or on a separate day. In all cases, motor activity testing will be done prior to dosing for the day and the temporal relativity with enhanced clinical observation testing will be the same for all groups.

Ophthalmic Examinations
Prior to treatment and during Week 3, an examination will be performed by a board-certified veterinary ophthalmologist on all animals using an indirect ophthalmoscope. The eyes will be dilated with a mydriatic agent prior to examination. Examination of the eye will include the anterior portion (eyelids, conjunctiva, cornea, anterior chamber, iris, and lens), the optic media (vitreous) and the ocular fundus. The ophthalmic examinations will be done on days other than those scheduled for the expanded clinical observations. Animals will be examined in a random order during Week 3 and the ophthalmologist will be unaware of each animal's dose level.

Dermal Irritation
Dermal irritation will be scored on Day 1 and weekly thereafter (including the day of necropsy), prior to dose application. Scoring will be done according to the scale in Attachment No. 2

Body Weights
At arrival, once for randomization, on the first day of treatment, and weekly thereafter

Food Consumption
Weekly

Clinical Pathology

Frequency
Blood and urine will be collected from all animals before the terminal sacrifice. When possible, blood will be collected from animals (non-fasted) sacrificed at unscheduled intervals.

Number of Animals
All

Method of Collection
Animals will be fasted overnight and urine will be collected on wet ice overnight and refrigerated until analyzed. Blood will be collected from a jugular vein.

The anticoagulants will be sodium citrate for the coagulation tests and potassium EDTA for the hematology tests. Samples for clinical chemistry will be collected with no anticoagulant.

Tests

Hematology

red blood cell (erythrocyte) count	platelet count
hemoglobin	white blood cell (leukocyte) count
hematocrit	differential leukocyte blood cell count
mean corpuscular volume	blood cell morphology
mean corpuscular hemoglobin	reticulocyte smear (made but not examined)
mean corpuscular hemoglobin concentration	

Two blood smears for differential leukocyte blood cell counts will be prepared and stained for each animal.

Reticulocyte smears will be evaluated only if there are group differences in the measurements on the erythron or leukon as determined by a pathologist and after consultation with the sponsor (to be added by amendment).

Coagulation (Terminal Sacrifice Only)

prothrombin time	activated partial thromboplastin time

Clinical Chemistry

glucose	alanine aminotransferase
urea nitrogen	gamma glutamyltransferase
creatinine	alkaline phosphatase
total protein	aspartate aminotransferase
albumin	calcium
globulin	inorganic phosphorus
albumin/globulin ratio	sodium
cholesterol	potassium
triglycerides	chloride
total bilirubin	

Urinalysis

specific gravity	blood
pH	urobilinogen
protein	volume (approximately 16 hours)
glucose	microscopic examination of sediment
ketones	appearance
bilirubin	

Termination

Unscheduled Sacrifices and Deaths

Necropsies will be done on all animals that die or are sacrificed in moribund condition. If possible, blood will be collected for clinical pathology tests from animals sacrificed at unscheduled intervals. Animals to be sacrificed will be anesthetized with an intraperitoneal injection of sodium pentobarbital and exsanguinated.

Terminal Sacrifice

After at least 21 days of treatment, all surviving animals will be fasted overnight, and blood will be collected for clinical pathology tests from a jugular vein. Fasted animals will be anesthetized with an intraperitoneal injection of sodium pentobarbital, weighed, exsanguinated, and necropsied. Animals will be necropsied in random order to eliminate temporal and technical bias. Random order will include, when possible, an approximate equal distribution of animals/sex/group. A board-certified veterinary pathologist will be present at each scheduled necropsy.

Postmortem Procedures

Necropsy

The necropsy will include an examination of the external features of the carcass; external body orifices; the abdominal, thoracic, and cranial cavities; organs; and tissues.

Organ Weights
At scheduled sacrifice, the following organs (when present) will be weighed; paired organs will be weighed together.

adrenal (2)	pituitary
brain	prostate
epididymis (2)	spleen
heart	testis (2)
kidney (2)	thymus
liver	thyroid (2) with parathyroid
ovary (2)	uterus

Organ-to-body weight percentages and organ-to-brain weight ratios will be calculated.

Bone Marrow Smears
From the femur of all animals at scheduled necropsy; made but not examined, unless requested by the sponsor (to be added by amendment)

Tissue Preservation
The following tissues (when present) from each animal will be preserved in 10% neutral-buffered formalin, unless otherwise specified.

adrenal (2)
aorta
brain (cerebrum, cerebellum, and medulla)
cecum
cervix
colon [proximal and distal (2)]
duodenum
epididymis (2)
esophagus
eye [(2) including retina and optic nerve]
femur with bone marrow (articular surface of the distal end)
Harderian gland
head[a]
heart
ileum (including Peyer's patch)
jejunum
kidney (2)
lacrimal gland (exorbital)
liver
lung with mainstem bronchi
lymph node (mandibular and mesenteric)
mammary gland (females only)
ovary (2)
pancreas
pituitary
prostate
rectum
salivary gland [mandibular (2)]
sciatic nerve
seminal vesicle (2)
skeletal muscle (thigh)
skin (treated and untreated)
spinal cord (cervical, thoracic, and lumbar)
spleen
sternum with bone marrow
stomach (nonglandular and glandular)
testis [preserved in Bouin's fixative for sacrificed animals (2)]
thymus
thyroid (2) with parathyroid
tissues with macroscopic changes or alterations (i.e., gross lesions)
tongue
trachea
urinary bladder
uterus with uterine horns
vagina

a The head (including pharynx, larynx, and nose) will be preserved in 10% neutral-buffered formalin for possible future examination.

Histopathology

Tissues (excluding the testes and epididymides) from each animal in the control and high-dose groups and each animal that dies or is sacrificed at an unscheduled interval will be embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically by a board-certified veterinary pathologist.

Any observed lesions from animals in any group will be collected, processed, and examined microscopically. Suspected target organs noted at the high dose will be examined microscopically from each animal (at the sponsor's request and added by amendment).

Following collection from each male (Groups 1 through 4), the epididymides will be separated from the testes. The tunic of each testis will be nicked at the caudal end to facilitate fixation, the left and right testes will be weighed together, and then placed in Bouin's fixative. The epididymides will be weighed together. After weighing, the epididymides will be incised before being fixed in 10% neutral-buffered formalin. After adequate fixation, each testis will be sectioned transversely in half, and the cranial pole will be sectioned for routine embedding in paraffin, histologic processing, and staining with hematoxylin and eosin. The remaining testicular tissue will be stored in 70% ethyl alcohol. The epididymides will be sectioned longitudinally and processed routinely for histologic evaluation. This longitudinal section should ensure that the head and mid portion of the epididymides are evaluated histologically. The remaining portions of the epididymides will be stored in 10% neutral-buffered formalin. Testes and epididymides from each male in the control and high-dose groups (Groups 1 and 4) from the terminal sacrifice and each male that dies or is sacrificed at an unscheduled interval will be examined microscopically by a board-certified veterinary pathologist.

Pathology Peer Review

The sponsor or sponsor's designee will perform a peer review of microscopic findings. The peer review will be performed according to the sponsor's SOPs. The draft pathology report, individual macroscopic and microscopic animal data, and tissue slides will be sent to the sponsor for review. Documentation of the peer review, including a peer review certificate, and the tissue slides will be returned to Covance following the peer review. The study director will receive a copy of the peer review certificate. The slides, draft pathology report, and macroscopic and microscopic animal data will be shipped to:

Sue Stitzel
Miami Valley Laboratories
Procter & Gamble Company
11810 East Miami River Road
Cincinnati, OH 45252
Telephone No.: 513.627.2893
Facsimile No.: 513.627.0390

The recipient will be notified before shipment.

Reports

Final Report

Four copies of the audited draft final report including, but not limited to, the items listed below will be submitted to the sponsor. The report will contain all elements required under the regulatory compliance section of the protocol.

Experimental Design and Methods

Results

dose analysis
mortality (to include fate and date of death for each animal)
clinical observations
expanded clinical observations
motor activity counts (mean 2-minute interval counts, 10-minute cumulative counts, and total counts)
ophthalmic observations
dermal irritation
body weights
body weight changes
food consumption
clinical pathology results
organ weight data
macroscopic observations
microscopic observations

Statistical Evaluation

Levene's test will be done to test for variance homogeneity. In the case of heterogeneity of variance at $p \leq 0.05$, transformations will be used to stabilize the variance. Comparison tests will take variance heterogeneity into consideration.

One-way analysis of variance (ANOVA) will be used (if applicable) to analyze body weights, body weight changes, food consumption, total motor activity counts, grip strength, nociceptive reflex, continuous clinical pathology data, and organ weight data. If the ANOVA is significant, Dunnett's t-test will be used for control versus treated group comparisons.

If the ANOVA shows significance for body weights at Week 1, one-way analysis of covariance (ANCOVA) will be used to analyze body weights, with initial body weights as the covariate. If the ANCOVA is significant, covariate-adjusted means will be used for group comparisons.

Expanded clinical observation categorical data will be analyzed by contingency table methods appropriate for the type of response measured (e.g., binary, such as present or absent piloerection; ordinal, such as reactivity to handling; nominal with more than two levels, such as gait description). Methods described by Agresti and implemented in StatXact software will be used.

Group comparisons (Groups 2 through 4 versus Group 1) will be evaluated at the 5.0%, two-tailed probability level. Only data collected on or after the first day of treatment will be analyzed statistically.

No-Effect/No-Adverse-Effect Level, Discussion, and Conclusion
The report will include a no-effect or no-adverse-effect level and a discussion and interpretation of the results.

One year after issuance of the audited draft report, if no requested revisions or instructions to finalize have been communicated by the sponsor, the audited draft report will be issued as the final report, signed by the study director, and submitted to the sponsor. Any modifications or changes to the audited draft report requested 1 year after issuance will be performed at additional cost to the sponsor.

Four copies of the audited draft report and four copies of the final report will be shipped to:

Ms. Cherie Maddin
The Procter & Gamble Company
Miami Valley Laboratory
11810 East Miami River Road
Cincinnati, Ohio 45252

Record Retention
The raw data, documentation, records, specimens, protocol, and final report generated as a result of this study will be archived in the storage facilities of Covance-Madison. At least 6 years after submission of the final report, the Covance Archives staff will contact the sponsor. At that time, the sponsor may choose to have the aforementioned materials returned, archived for an additional period of time, or disposed of by Covance; a fee will be charged based on the archive disposition option selected by the sponsor. Raw data stored on magnetic media and the protocol, study correspondence, and the original final report will be retained by Covance.

Tissue specimens (wet and in paraffin) and tissue (including bone marrow slides) and blood slides will be sent to Pathology Associates International, West Chester, Ohio, after the report is finalized (Attachment No. 3).

Protocol Changes
Changes to the protocol will be made by amendment after consultation by the study director and study monitor. The amendment will state the reason for the change.

PROTOCOL APPROVAL

Jennifer L. Counts, PhD
Study Monitor
The Procter & Gamble Company

27 NOV 2000
Date

Susan M. Henwood, MS
Diplomate ABT
Study Director
Covance Laboratories Inc.

20 November 00
Date

Attachment No. 1

Expanded Clinical Observations

Variations in the test conditions will be minimized to avoid systematically relating to treatment group.

Assessments will be made upon removal of the animal from the cage (Hand-held Observations) and in a standard circular arena (Open Field Observations). After the Open Field Observations, a battery of manipulative tests will be performed (Elicited Behaviors).

Hand-held Observations

1. Reactivity to handling. The technician removes the animal from its home cage and makes a subjective assessment of the reactivity (e.g., squirming) to handling.
   - ☐ No reactivity
   - ☐ Low reactivity
   - ☐ Moderate reactivity
   - ☐ High reactivity

2. Vocalization
   - ☐ No vocalization
   - ☐ Vocalization upon removal only
   - ☐ Vocalization during handling only
   - ☐ Vocalization during removal and handling

3. Palpebral closure. The technician observes the degree of closure of the eyelids.
   - ☐ Eyelids wide open
   - ☐ Ptosis
   - ☐ Eyelids completely closed

4. Exophthalmos. The technician observes the protrusion of the eyeballs.
   - ☐ Absent
   - ☐ Present

5. Excessive lacrimation. The technician observes the presence of excess wetness around the eye.
   - ☐ None
   - ☐ Small amount
   - ☐ Moderate amount
   - ☐ Large amount

Attachment No. 1 (Continued)

6. Excessive salivation. The technician observes the presence of excess wetness around the mouth.
   - ☐ None
   - ☐ Small amount
   - ☐ Moderate amount
   - ☐ Large amount

7. Respiration
   - ☐ Appropriate for air exchange
   - ☐ Abnormal (describe: e.g., rapid and shallow, labored, gasping)

8. Appearance of fur. The technician observes the condition of the animal's fur over the entire body.
   - ☐ Groomed
   - ☐ Stained (describe appearance and location)

9. Piloerection
   - ☐ Absent
   - ☐ Present

10. Muscle tone. The technician supports the subject in the air by grasping the thorax gently from behind. With the free hand, the technician gently but briskly presses the tips of two fingers into the middle of the plantar surface (footpads) of each hindlimb. As the animal extends the hindlimbs, the presence/strength of the extensor thrust response is evaluated subjectively via digital palpation. Several presses may be required to elicit the extensor response.
    - ☐ None
    - ☐ Low
    - ☐ Moderate
    - ☐ High

11. Pupillary status (relative to room lighting conditions). The technician observes the status of the pupils.
    - ☐ Miosis
    - ☐ Normal
    - ☐ Mydriasis

Attachment No. 1 (Continued)

Open Field Observations

Each animal will be placed into an open field arena for approximately 2 minutes. The open field will be large enough for the animal to walk freely so that the observer can

evaluate gait. The open field arena will provide an unobstructed view of the animal. Animals will be observed for the following (any unusual observations will be described as they are observed):

12. Locomotor activity. The technician observes and makes a subjective assessment of the amount of motor activity (e.g., horizontal and vertical activity).
    - ☐ None
    - ☐ Low
    - ☐ Moderate
    - ☐ High

13. Posture. The technician observes the animal's posture and records the observations.
    - ☐ Typical range of postures (e.g., sitting, rearing, walking)
    - ☐ Other (describe: e.g., low carriage, hunched, prostrate)

14. Gait Abnormalities - type and severity. The technician observes the animal's gait. If gait abnormalities are present, the severity is recorded as slight, moderate, or severe.
    - ☐ None
    - ☐ Other (describe: e.g., ataxia, retropulsion)

15. Other unusual behavior
    - ☐ Absent
    - ☐ Present (describe and rank if appropriate)

Attachment No. 1 (Continued)

Elicited Behaviors

Each animal will be tested for auditory reactivity before removal from the open field arena. Following these tests, each animal will be removed from the open field arena and evaluated for all subsequent elicited behaviors while the technician gently restrains or holds the animal.

16. Auditory reactivity. The technician brings a clicking device, from behind the animal, approximately one inch above the head. The clicking device will then be activated and the technician will observe the animal's reaction. The response will be recorded.
    - ☐ No visible flinch
    - ☐ Visible flinch
    - ☐ Greater than typical flinch

17. Proprioceptive Positioning Reaction. The technician gently restrains the subject on a horizontal surface by grasping the thorax. The hindlimb to be tested is grasped and repositioned so that the dorsal surface of the paw is on the testing surface. The technician relaxes the grasp of the hindlimb. The animal will normally return the hindpaw to normal weight bearing position quickly. The test may be repeated several times and a representative performance will be reported.
    - ☐ Limb not returned to weight-bearing position
    - ☐ Limb slowly returned to weight-bearing position
    - ☐ Limb quickly returned to weight-bearing position

18. Pinna response. The technician holds the animal in one hand. With the free hand, the technician lightly touches the inner surface of one external ear near the ear canal with a fine probe. This test may be repeated on the same or opposite ear.
    - ☐ No response
    - ☐ Ear is flattened, animal shakes head, or both

19. Pupillary status. The technician holds the animal in his/her hand in a dimly lit area of the room and holds a penlight in the free hand. Just before shining the penlight into each eye of the animal in succession, the technician observes the status of the pupils.
    - ☐ Miosis
    - ☐ Normal
    - ☐ Mydriasis
    - ☐ No change in pupils in black box

Attachment No. 1 (Continued)

20. Pupillary response. The technician holds the animal in one hand in a dimly lit area of the room and, with the free hand, shines a penlight into each eye of the animal in succession. The technician observes the responses of the pupils.
    - ☐ Neither pupil constricts
    - ☐ Left pupil constricts
    - ☐ Right pupil constricts
    - ☐ Both pupils constrict
    - ☐ Unable to assess

21. Grip strength. Animals will be tested using an apparatus consisting of a grasping bar attached to a strain gauge. The apparatus will be operated and calibrated according to standard operating procedure. The animal will be allowed to grip the forelimb grasping bar, and is pulled away from the bar until grip is broken. Then the animal will be held so its hindlimbs grip the hindlimb grasping bar and is pulled away from the bar until grip is broken. Grip strength will be recorded for both forelimb and hindlimb grip. Three trials each for the forelimbs and hindlimbs will be done. The results of each trial will be recorded in grams of force and the median grip strength value of the three trials will be used as the animal's score.

22. Nociceptive reflex. The animal's tail will be placed onto an automated system. A heat stimulus is applied to the tail. The time (in seconds) for the tail to move away from the nociceptive stimulus will be recorded.

Attachment No. 2

Irritation Scoring Scale[a]

Erythema (redness of the skin)

0 No erythema
1 Very slight erythema (barely perceptible)
2 Well-defined erythema
3 Moderate to severe erythema
4 Severe erythema (beet redness) to slight eschar formation (injuries in depth)

Edema (swelling of the skin)

0 No edema
1 Very slight edema (barely perceptible)
2 Slight edema (edges of area well-defined by definite raising)
3 Moderate edema (raised approximately 1 mm)
4 Severe edema (raised more than 1 mm and extending beyond area of exposure)

Atonia (impairment of elasticity)

No
Yes

Desquamation (scaling and flaking)

No
Yes

Fissuring (definite cracks in the dermis)

No
Yes

Eschar (injuries in depth, possible necrosis)

No
Yes

a Grades assigned will be based on the most severely affected area.

Note: This scale is a modified presentation of the original Draize

Draize, J. H., *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics,* pp. 46-49, The Association of Food and Drug Officials of the United States, Austin, TX (1959).

Attachment No. 3

Instructions for Return of Specimens, Wet Tissues, Paraffin Blocks, and Microscopic Slides for Archiving for The Procter & Gamble Company

Wet Tissues
Wet tissues must be heat-sealed in plastic bags. Kapak Pouches by Skotchpak or equivalent bags will be used. Place only one animal's tissues in a bag together with an amount of 10% neutral-buffered formalin or other fixative adequate to thoroughly moisten all tissues. An identifying label with the study number and specimen identification number written with pencil or indelible marker should be included in the bag with the wet tissues. Several seals should be made, and an identifying label with the study number and specimen identification number written with waterproof medium should be placed between the seals. This bag will be placed in a second bag and the second bag will be heat sealed. Double-bagged tissues will be packed in plastic-lined cardboard boxes. The maximum size of the box should not exceed 17.5 x 10 x 8 inches. The exterior of the box should be labeled with the study number, range of inclusive specimen identification numbers, and box number.

Tissue Blocks
Paraffin blocks should be placed on end in numerical order by group number (when applicable) with the label visible showing the specimen identification number. In some instances, paraffin blocks may require heat-sealing; this is performed at the discretion of the histology laboratory. Cardboard trays measuring 16 x 9 x 2 inches and protective cardboard sleeves or similar boxes will be used. The exterior of the box should be labeled with the study number and range of inclusive specimen identification numbers.

Microscope Slides
All microscopic slides (bone marrow, blood smear, and histopathologic, as applicable) will be submitted for archiving. The slides should be packaged in numerical order by groups (when applicable) in plastic boxes designed to hold 100 glass microscope slides with a minimum of movement within the box. The slide boxes should be secured with tape and wrapped in shock-absorbent materials before placed into a shipping box. Slides must be labeled with the study number and specimen identification number. The identification must be indelible to preclude loss of identity during handling. Pencil is not acceptable.

All tissue specimens will be sent to the sponsor's representative for archiving at the following address.

Pathology Associates International
6217 Centre Park Drive
West Chester, Ohio 45069
Attn: Brad Peterson
Telephone No.: 513.779.9600

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

PROTOCOL AMENDMENT NO. 1

Covance 6114-398
Procter & Gamble Study No.: DRD: SSBTS00.040-52068

21-Day Dermal Toxicity Study with SS0853.01 in Rats

Sponsor:	The Procter & Gamble Company, Cincinnati, Ohio.
Study Monitor:	Jennifer L. Counts, PhD
Testing Facility:	Covance Laboratories Inc., Madison, Wisconsin
Study Director:	Susan Henwood, MS, DABT

This amendment modifies the following portions of the protocol.

Effective 27 November 2000

1. **Animals, Weight at Initiation of Treatment.** To reflect the body weight range of animals selected for use on study, delete the text in this section and replace with the following.

   Females: 162 to 217 g
   Males: 260 to 320 g

2. **Dose Analysis, Routine Analysis, Sentences 1 and 2.** To reflect that the high-dose level is dosed as the neat test material and therefore does not require concentration analysis, delete these sentences and replace with the following.

   Samples (approximately 5 mL each) from the low- and mid-dose preparations will be collected and analyzed. Stability samples collected from the low- and mid-dose level preparations and analyzed on the day of mixing will be used for Week 1 routine analyses.

AMENDMENT APPROVAL

Jennifer L. Counts, PhD
Study Monitor
The Procter & Gamble Company

Dec. 5, 2000
Date

Susan M. Henwood, MS
Study Director
Covance Laboratories Inc.

01 Dec 00
Date

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

PROTOCOL AMENDMENT NO. 2

Covance 6114-398
Procter & Gamble Study No.: DRD: SSBTS00.040-52068

21-Day Dermal Toxicity Study with SS0853.01 in Rats

Sponsor:	The Procter & Gamble Company, Cincinnati, Ohio
Study Monitor:	Jennifer L. Counts, PhD
Testing Facility:	Covance Laboratories Inc., Madison, Wisconsin
Study Director:	Susan Henwood, MS, DABT

This amendment modifies the following portion of the protocol.

Effective 02 January 2001

1. **Experimental Design and Methods, Statistical Evaluation.** To reflect a change in the statistical analysis procedures to be used on this study, delete the text in this section and replace with the following.

   The observed values for body weights, body weight changes, food consumption, motor activity counts, grip strength, nociceptive reflex, continuous clinical pathology data, and organ weight data will be evaluated statistically.

   If Levene's test for variance homogeneity is not significant ($p>0.05$), one-way analysis of variance (ANOVA) will be performed on the observed values. If Levene's test is significant ($p\leq 0.05$), ANOVA will be done on the rank transformed data. *Post hoc* Dunnett's t-test will be used for control vs. treated group mean comparisons, incorporating the transformation when necessary.

   Expanded clinical observation categorical data will be analyzed by contingency table methods appropriate for the type of response measured (e.g., binary, such as present or absent piloerection; ordinal, such as reactivity to handling; nominal with more than two levels, such as gait description). Methods described by Agresti and implemented in StatXact software will be used.

   Control versus treated group comparisons will be evaluated at the 5.0%, two-tailed probability level. Only data collected on or after the first day of treatment will be analyzed statistically. Data for each sex will be analyzed separately.

AMENDMENT APPROVAL

Jennifer L. Counts, PhD
Study Monitor
The Procter & Gamble Company

16 Jan 2001
Date

Susan M. Henwood, MS
Study Director
Covance Laboratories Inc.

16 January 2001
Date

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

PROTOCOL AMENDMENT NO. 3

Covance 6114-398
Procter & Gamble Study No.: DRD: SSBTS00.040-52068

21-Day Dermal Toxicity Study with SS0853.01 in Rats

Sponsor:	The Procter & Gamble Company, Cincinnati, Ohio
Study Monitor:	Jennifer L. Counts, PhD
Testing Facility:	Covance Laboratories Inc., Madison, Wisconsin
Study Director:	Susan Henwood, MS, DABT

This amendment modifies the following portions of the protocol.

Effective 08 January 2001

1. To account for the acquisition of Pathology Associates International (PAI) by Charles River Laboratories, replace all occurrences of "Pathology Associates International" in the protocol, with the following.

   Pathology Associates, A Charles River Company

Effective 02 August 2001

2. **Study Monitor.** To reflect a change in the study monitor assigned to this study, delete the text in this section and replace with the following.

   Rob Stachlewitz, PhD
   The Procter & Gamble Company
   Telephone No.: 513.626.1665
   Facsimile No.: 513.626.3522

AMENDMENT APPROVAL

Rob Stachlewitz, PhD
Study Monitor
The Procter & Gamble Company

13 August 2001
Date

Susan M. Henwood, MS
Study Director
Covance Laboratories Inc.

07 August 2001
Date